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# Role of *Samyoga* and *Samskara* on Anti-hyperlipidaemic / Anti-obesity activity of Honey – An Experimental Study

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## Abstract

**Background:** *Madhu* (honey) is conventionally used food and medicine since ages. Ayurvedic literatures emphasizes implication of *Madhu* in the management of *Kaphaja Vyadhi* (disease caused by vitiated phlem) and *Sthoulya* (obesity) due to *Medo Dhatu Vruddhi* (increase in adipocyte mass). **Aim:** To evaluate Anti-hyperlipidaemic and anti-obesity activity of *Madhu*. **Materials & Methods:** The present experimental study was conducted on high fat diet fed Wistar albino rats. *Madhu* mixed with *Triphala Kashaya* (*Samyoga* - added) and processed with *Triphala Kashaya* (*Samskara* - processed) was administered for 48 days (One *Mandala Kala*). Reduction in body weight, blood lipid profile and organ weight were recorded and compared statistically with the mean values of groups treated with *Kevala Madhu* (pure honey) and *Jala Samskarita Madhu* (honey processed with water). **Results:** Reduction in body weight was observed in all treated groups. *Triphala Kashaya Samskarita Madhu* treated animals exhibited remarkable reduction in body weight, serum triglycerides, total cholesterol, LDL, VLDL and elevated HDL levels. Changes pertaining to vital organ weight (liver, heart and kidney) were also corresponding with reduction in body weight of treated group animals. **Conclusion:** The study revealed significant anti-hyperlipidaemic / anti-obesity activity of *Samskarita Madhu*.

**Keywords:** *Anti-hyperlipidaemic activity, Anti-obesity activity, Honey, Samskara, Samyoga, Triphala*

## Introduction

Obesity, a medical condition involving excess accumulation of body fat has become a major disorder throughout the world [1]. The International Obesity Task Force estimates that more than 300 million individuals worldwide are obese and an additional 800 million are overweight [2]. In 21st century, obesity has reached epidemic proportions in India with morbid obesity affecting 5% of the country's population[3].

*Sthoulya* (obesity) is a condition enumerated under *Ashtanindhitha Purusha* (eight unsuitable category of people) [4], which is characterized by excess accumulation of *Medas* (fat)[5] resulting in complications like *Ayushyahrasa* (decreased life span), *Dhoouurgandhya* (foul smell), *Vyavayakrichratha* (reduced libido) and *Dhourbalya* (debility)[6]. *Sthoulya Chikitsa* (treatment of obesity) comprises of *Sleshma* and *Medohara Karma* (removing phlegm & lipolysis) with *Rooksha* and *Ushna Dravyas* [7] (drugs bringing about dryness and thermogenic).

Modern treatment modalities for obesity include lifestyle management and pharmacotherapy[8], which may have untoward effects such as congestive cardiac failure, hypertension and Coronary heart disease[9]. *Purana Madhu* (matured honey), one among *Medohara Dravyas*[10], has been indicated in *Sthoulya Prakarana* in its different forms. One among those is *Pakwa* or *Samskarita Madhu* with *Triphala Kashaya*.

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*Acharya* Kaiyadeva has attributed different techniques of *Madhupaka* to get specific outcomes. One among them is *Madhupaka* with *Triphala Kashaya* in the management of *Sthoulya*[11]. *Acharya* Bhavamishra has also mentioned usage of *Madhu Mishrita Triphala Kashaya* for *Medohara* activity [12].

With this perspective, the present study was conducted to evaluate *Medohara Karma* of *Madhu* with respect to its *Samyoga* and *Samskara* in high fat diet fed experimental animals.

**Materials and Methods:**

**Source of drug:**

Freshly extracted un-processed honey was obtained from the Government Honey production Society of Kanniyakumari district, TamilNadu and was stored in dry amber coloured glass bottles for one year to make it *Purana*. Deseeded fruits of *Haritaki* (*Terminalia chebula, Combrataceae*), *Vibhitaki* (*Terminalia bellarica, Combrataceae*) and *Amalaki* (*Emblica officinalis, Euphorbiaceae*) were procured from local market. Deseeded fruits were pounded using *Khalwa Yantra* (mortar and pestle) to obtain *Yavakuta* (coarse powder) *Triphala Choorna*. *Triphala Kashaya* was prepared as per standard protocol of Sharangadhara [13].

*Madhupaka Vidhi* (processing honey) was carried out using *Madhu* and *Triphala Kashaya* in equal proportions as per the reference of Kaiyadeva Nighantu[14] with minor modifications. Instead of heating *Madhu* directly over the flame, water bath (100$^0$C) was used during condensation process to avoid charring of sugar content. Same procedure was followed for the preparation of *Jala Samskarita Madhu* [15]. Samples of *Purana Madhu*, *Triphala Kashaya Samskarita Madhu* (processed with *Triphala* decoction) and *Jala Samskarita Madhu* (Processed with water) were analysed for moisture content, mineral content, pH, specific gravity and HMF (Hydroxy methyl purfurol) contents at Sigma test and Research centre New Delhi.

**Preparation of High Fat Diet:**

High fat diet [16] for Wistar albino rats was prepared under standard laboratory conditions. Ingredients of diet (**Tabe** 1) were mixed, converted into pallets, dried in hot air oven and stored in cool and dry container.

**Experimental study:**

Ethical clearance was obtained from JSS Ayurveda Medical College, Mysore and JSS College of Pharmacy, Mysore (IEAC 164/2014) prior to commencement of the experimental study. Healthy Wistar Albino Rats weighing between 150 – 200 grams were procured from Animal Breeding Facility, Department of Pharmacology, JSS college of Pharmacy, Mysore.

**Table** 1 Ingredients of high fat diet

| S.No. | INGREDIENTS | WEIGHT grams/kg |
|---|---|---|
| 1. | Milk Powder | 100 |
| 2. | Wheat Flour | 610 |
| 3. | Hydrogenated vegetable oil | 170 |
| 4. | Sugar | 50 |
| 5. | Mineral Mixture | 40 |
| 6. | Vitamins | 20 |
| 7. | Fiber | 10 |

Experimental animals were sorted in 7 groups comprising 6 animals in each group (Table 2). Prior to commencement of experimentation, experimental animals were acclimatized for 15 days under standard laboratory conditions. Regular rat feed and potable water was provided during this period.

As there are no previous references available regarding duration of the study, 1 *Mandala Kala* (48 days) was considered[17]. Except control group animals, other groups received high fat diet ad libitum throughout the study period. Control group animals received regular rat feed throughout the study period. Administration of high fat diet and test drug was carried out simultaneously. The dose of test drug was determined and carried out as per the earlier study[18] as *Avaleha Pramana* [19]. Effective dose for the rat was calculated based on the dose conversion formula[20]. 860 mg/200g was fixed as initial dose and calculated periodically based on change in body weight during study period. Distilled water was used as media to administer *Samskarita Madhu*, whereas Group 6 animals received *Madhu* mixed with *Triphala Kashaya* (*Samyoga*). Group 4 and 6 animals received *Madhu* with 2 ml *Triphala Kashaya*. Body weight of the animals was recorded before commencement of experiment and on 10th, 20th, 30th, 40th and 48th day. Blood was collected from retro-orbital area on 48th day and resultant serum was subjected for lipid profile estimation. On 49th day, all experimental animals were sacrificed and vital organs obtained were weighed. Data was statistically analyzed by ANOVA method using Graph pad Prism version 6 software for level of significance.

**Table 2 Showing details of experimental groups**

| Sl.no | Groups | Diet & Drug Schedule of the groups |
|---|---|---|
| 1 | Group 1 | Control Group |
| 2 | Group 2 | High fat Diet +Untreated group |
| 3 | Group 3 | High fat Diet+ treated with *Triphala Kashaya* |
| 4 | Group 4 | High fat Diet+ treated with Purana *Madhu* |
| 5 | Group 5 | High fat Diet+ treated with Jala *Samskarita Madhu* |
| 6 | Group 6 | High fat Diet + treated with *Triphala Kashaya* and *Madhu* (*Samyoga*) |
| 7 | Group 7 | High fat Diet +Treated with *Triphala Kashaya Samskarita Madhu* |

## Observations and Results:

Results pertaining to Physico-chemical analysis are depicted in Table 3. Slight increase in HMF content was recorded in processed honey, which remained well within standard limits[21]. Flavonoids and phenolic concentrations increased slightly in processed honey where as pH and specific gravity remained relatively low. *Triphla Kashaya Samskarita Madhu* exhibited marked anti-oxidant activity compared to unprocessed and *Jala Samskarita Madhu*.

All animals pertaining to 7 groups remained healthy with normal food and water intake and no abnormal behaviour was noticed.

**Table 3 (Analysis of Honey before and after processing)**

| Samples | pH | Specific gravity | HMF Content (mg/kg) |
|---|---|---|---|
| Unprocessed honey | 4.16 ± 0.4 | 1.39 ± 0.14 | 19.19 mg/kg |
| Processed honey in water | 3.92 ± 0.4 | 1.41 ± 0.13 | 25.19 mg/kg |
| Processed honey with *Triphala Kashaya* | 4.64 ± 0.4 | 0.80 ± 0.08 | 27.61 mg/kg |

## Body weight:

Increase in body weight was noticed in control (Group 1) and High fat diet (Group 2) animals during study period (Table 4). High fat diet animals gained weight rapidly compared to control group animals. Marked decline in body weight was observed in all treated group animals among which *Triphala Kashaya Samskarita Madhu* (Group 7) treated animals showed reduction on 20th day itself compared to other treated groups. Reduction in body weight among *Samkarita Madhu* group (Group 5 and group 7) was statistically highly significant (P<0.001).

## Plasma lipid profile and vital organ weight:

High fat diet increased plasma total cholesterol, triglycerides, LDL and VLDL and decreased HDL levels among High fat diet group (Group 2). All treated group animals showed reduction in Total cholesterol, triglycerides, LDL and VLDL levels and increase in HDL levels (Table 5). Among all treated groups, TK *Samskarita Madhu* treated animals exhibited marked decline in LDL and VLDL levels and increased HDL levels which is Statistically highly significant (P<0.001).

## Discussion:

*Madhu* is considered as the ideal substance for weight management[22]. Many Experimental and clinical studies have proved effectiveness of *Madhu* for weight reduction[23]. Concept of honey turning in to poison due to heating process is highlighted in Ayurvedic literature and therefore needs careful evaluation for its safety and efficacy. Experimental studies can give inputs regarding toxicity and pharmacological activities. Honeybees collect nectar from various flowers including poisonous ones and occurrence of Clostridium botulinum spores have also been reported in earlier studies[24]. Such risks necessitate usage of processed/pasteurized honey, which destroy microbial contaminants and make honey safe for consumption[25]. Honey is regarded to be having synergistic activity when used along with other substances as per earlier studies conducted and same may be applicable for anti-hyperlipidaemic activity[26]. This quality can be attributed to the concept of *Yogavahitwa* (Synergistism) of *Madhu* described in treatises. *Samskara* (processing) is said to bring about changes in *Guna* and hence *Karma* is also affected.

**Table 4 Showing the effect of experimental group drugs on Mean Body weight**

| Sl No | Group | Day 0 | Day 10 | Day 20 | Day 30 | Day 40 | Day 48 |
|---|---|---|---|---|---|---|---|
| 1 | Control | 200.83±7.57 | 202.33±7.3 | 203.17±7.56 | 205.0±7.41 | 206.17±7.0 | 206.67±6.67* |
| 2 | Fat diet- control | 174.17±8.7 | 179.83±7.62 | 187.17±6.81 | 192.17±6.86 | 201.17±6.96 | 206.67±6.79* |
| 3 | *Triphala Kashaya* | 162.50±3.82 | 159.67±3.36 | 158.0±3.29 | 153.33±3.47* | 149.33±3.56* | 145.00±3.65* |
| 4 | *Purana Madhu* | 180.83±10.2 | 172.83±8.14 | 167.50±7.25 | 153.0±5.8* | 131.17±3.73* | 122.50±3.09** |
| 5 | *Jala Samskarita Madhu* | 185.0±10.49 | 177.17±10.54 | 170.0±9.28 | 145.00±9.4* | 131.50±9.63* | 125.0±8.76** |
| 6 | *Triphala Kashaya Samyoga Madhu* | 162.50±3.82 | 159.17±3.1 | 155.17±3.55 | 141.67±5.4* | 135.0±5.48* | 131.67±5.27** |
| 7 | *Triphala Kashaya Samskarita Madhu* | 180.83±11.5 | 170.67±9.3 | 157.67±10.02 | 135.67±6.96* | 117.83±6.6** | 105.83±6.77*** |

(Mean body weight expressed in grams ± SE at the intervals of 0, 10th, 20th, 30th, 40th and 48th day.
*P<0.05, **P<0.01, ***P<0.001)

**Table 5 Showing effect of experimental group drugs on Serum lipid profile**

| Sl No | Group | Total Cholesterol | Triglycerides | HDL | LDL | VLDL |
|---|---|---|---|---|---|---|
| 1 | Control | 91.33±3.69 | 82.67±1.5 | 36.83±1.08 | 56.0±1.07 | 32.17±0.87 |
| 2 | Fat diet – control | 127.50±2.6 | 143.83±2.21 | 28.17±1.54 | 74.0±1.48 | 45.33±1.11 |
| 3 | *Triphala Kashaya* | 70.83±2.43 | 67.83±1.42 | 41.17±1.62 | 47.83±1.01* | 22.50±0.76* |
| 4 | *Purana Madhu* | 63.67±1.43* | 57.83±1.14* | 46.17±1.14* | 38.0±1.07** | 23.33±1.36* |
| 5 | *Jala Samskarita Madhu* | 63.67±1.71* | 55.50±2.39* | 40.83±0.83 | 37.67±1.71** | 24.17±1.25* |
| 6 | *Triphala Kashaya Samyoga Madhu* | 58.17±2.2* | 48.50±1.48* | 44.17±1.42* | 34.50±1.06** | 20.50±1.52** |
| 7 | *Triphala Kashaya Samskarita Madhu* | 53.17±2.78* | 37.33±2.61*** | 48.17±2.23* | 31.33±1.05*** | 16.83±1.78*** |

(Mean values expressed in mg/dl ± SE. *P<0.05, **P<0.01, ***P<0.001)

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**Table 6 Showing effect of experimental group drugs on weight of vital organs**

| SI No | Group | Heart | Liver | kidney |
|---|---|---|---|---|
| 1 | Control | 0.56±0.01 | 4.94±0.24 | 1.04±0.05 |
| 2 | Fat diet - control | 0.74±0.03 | 5.28±0.19 | 1.28±0.1 |
| 3 | *Triphala Kashaya* | 0.52±0.01 | 4.41±0.15 | 0.93±0.02 |
| 4 | *Purana Madhu* | 0.47±0.02* | 4.24±0.1 | 0.87±0.02* |
| 5 | *Jala Samskarita Madhu* | 0.41±0.01* | 4.23±0.13 | 0.87±0.01* |
| 6 | *Triphala Kashaya Samyoga Madhu* | 0.44±0.02* | 4.11±0.07* | 0.83±0.01* |
| 7 | *Triphala Kashaya Samskarita Madhu* | 0.39±0.01** | 4.05±0.04* | 0.82±0.01** |

(Mean weight expressed in grams ± SE. *$P<0.05$, **$P<0.01$, ***$P<0.001$)

Caramelization and elevation in HMF content are the risks involved with heating process[27], which accelerates the reaction making honey unfit for consumption. In conventional system, temperature ranging between 72°C -110°C [28] is used in processing units.

*Madhu* is considered as *Ahara Upayogi Dravya* (dietary food substance) and hence specific quantity/dosage is not explained in Ayurvedic literature. Many clinical studies conducted on healthy as well as diabetic individuals using 40-50 g[29] honey per day have shown encouraging results and hence 48 g dose was considered for the present study, which corresponds with *Avaleha Pramana*. Un-processed honey has been evaluated for anti obesity effect clinically and experimentally with positive results [30]. Decrease in body weight among all treated group signify the *Medohara* activity of *Madhu* and *Triphala Kashaya*. *Madhu* being *Yogavahi Dravya*, might have accelerated the *Medohara Karma* of *Triphala Kashaya*, which is evident from present study.

Evidences available so far reveal about the nutrient oligosaccharide[31] and fructose content[32] of honey and their possible role in weight reduction. Furthermore honey is a source of phenolic compounds[33], which act as anti oxidants[34]. Studies on tannins and phenolics have also confirmed their possible anti nutrient effect[35]. Interference in digestion and functioning of digestive enzymes by phenolics lead to less absorption of carbohydrates, fats and proteins through intestine causing body weight reduction. Analysis of *Samskarita Madhu* has testified increase in phenolic and flavonoid contents. Effect of temperature during processing on enhanced activity of *Madhu* cannot be ruled out and hence needs detailed investigation.

*Madhu* and *Triphala* are said to be *Tridosha Shamaka*, i.e, maintenance of physiological status and health. which indicates the balancing activity of *Madhu* and *Triphala Kashaya* in metabolic like hyper lipidemia as it is reported even in non- obese person. Lipid profile, which include total cholesterol, Triglycerides, HDL, LDL and VLDL are maintained within normal range in all treated groups, where as high fat diet group animals showed significant increase in cholesterol and reduction in HDL levels. These findings also suggest the preventive aspect besides curative nature of *Madhu* in obesity. Thus daily consumption of *Madhu/Triphala Kashaya/Samskarita Madhu* may restore physiological status among human beings. Anti oxidant effect of phenolics must have played very important role in maintenance of lipid profile among all treated group animals [36].

Findings of earlier studies have revealed the beneficial effect of honey on liver and heart of experimental animals [37], which were analysed through enzymatic studies. Honey in its natural form and processed form may prevent accumulation of excess fat in vital organs,thus maintain their health, which is supported by the present study.

## Conclusion:

The present study clearly demonstrated that *Triphala Kashaya Samskarita Madhu* exhibits potential anti-obesity/anti-hyperlipidemic activity. *Madhu* in the form of both *Samyoga* and *Samskara* will be hence helpful in dealing with increasing rate of obesity. In future clinical studies have to be conducted to study efficacy of *Madhu* for weight management.

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